

Lecture Notes February 14, 2000

A) Review Vesicle cycle

Synaptic vesicle cycle - broken down into several steps: 1) subset of vesicles within the terminal are "docked" at the active zone; 2) Ca^{++} influx triggers some of these docked vesicles fuse with the plasma membrane; 3) the vesicle membrane and its associated proteins are then taken up by clathrin-mediated endocytosis; 4) synaptic vesicles are reformed and refilled with ACh; 5) vesicles are then targeted/clustered at active zones. Vesicles are then redocked (step 1) in preparation for another round.

B) Today - discuss some of the proteins involved in docking and/or fusion and how Clostridial toxins helped identify them.

Three separate lines of research all contributed vital information to this story:

1) Purification and cloning of nerve terminal proteins including: on the vesicle - synaptophysin, synapsin, synaptotagmin and VAMP (also called synaptobrevin); on the presynaptic membrane - neuexin, syntaxin, and SNAP-25. However, did not provide functional data.

2) Clostridial toxins

a) Effect neuromuscular function by blocking the release of neurotransmitters from presynaptic terminals. They don't effect vesicle docking and they don't effect the level of Ca^{++} entering the terminal - but they stop neurotransmitter release.

b) These toxins are made by anaerobic bacteria - Clostridium botulinum (7 types - BoNT A,B,C,D,E,F and G) produce the 7 different botulinal toxins which cause botulism, and Clostridium tetani produces tetanus toxin (TeTx) which causes tetanus. These toxins (particularly the BoNT's) are among the most potent toxins known.

c) The bacteria, or their spores, are everywhere - dirt, dust, and manure - and Clostridium botulinum is also found in lakes, streams, oceans, fish and mammals.

d) The bacteria require an oxygen-free environment in which to grow (anaerobic). Clostridium tetani accomplishes this by growing in any wound that provides an anaerobic environment - especially deep wounds or wounds contaminated with dirt. Clostridium botulinum tends to grow in improperly canned foods where it then produces significant amounts of toxin. It's the consumption of the toxin, not the bacteria, that produces it's often deadly effects (the bacteria do not survive in the normal adult digestive system).

e) The toxin is usually systemically distributed (although not always). Then it specifically binds to motor nerve terminals.

f) Clostridial toxins are made up of two peptides - a heavy chain and a light chain. The heavy chain specifically binds to receptors on motor nerve terminals permitting endocytosis of the toxin. The light chain mediates the toxicity.

Here the story for BoNT's and TeTx diverge.

g) BoNT's are targeted to the endosome, where the low pH environment causes dissociation of

the light chain which is somehow then released into the cytoplasm of the motor nerve terminal where it inhibits release of the neurotransmitter - causing paralysis.

h) TeTx is retrogradely transported back to the motor neuron cell body in the spinal cord. It is then transported to a population of inhibitory interneurons in the spinal cord where the light chain is somehow released into the terminal cytoplasm. TeTx prevents the release of inhibitory neurotransmitters onto motor neurons - also causing paralysis but of a different kind.

i) In botulism you get flaccid paralysis. Whereas in tetanus, you get spastic paralysis. So in Botulism - the effected muscles go limp because no neurotransmitter is released onto the muscle fiber, whereas in tetanus - the muscles are in uncontrolled contraction due to less inhibitory input onto the motor neurons.

j) The light chains are Zn-dependent metalloproteases that cleave presynaptic proteins. VAMP/syb is cleaved by TeTx, BoNT's B,D,F,G, SNAP-25 by BoNT's A & E, and syntaxin by BoNT C. Since the Clostridial toxins inhibit transmitter release without affecting vesicle docking or incoming Ca^{++} levels, this suggests that these three proteins are involved either in priming the vesicle for fusion or the fusion event itself. Figure 1.

3) The SNARE Hypothesis. (for SNAP REceptor) - see also Figures 14-15 of text

a) Cytoplasmic factors required for vesicle trafficking: NSF (for N-ethylmaleimide-Sensitive Factor) and a group of SNAP proteins (these SNAPS are absolutely unrelated to SNAP-25).

b) VAMP, syntaxin, and SNAP-25 bind to each other and form a "SNARE complex." Also frequently called a "core complex" or "fusion complex". Figure 2.

c) According to this hypothesis: VAMP and synaptotagmin on the vesicle membrane interact with syntaxin and SNAP-25 on the presynaptic membrane forming a stable protein complex that docks vesicles at release sites. Synaptotagmin appears to be mostly restricted to regulated release pathways. When Ca^{++} enters, synaptotagmin is proposed to move out of the way, allowing the fusion reaction to proceed. Most likely following fusion, SNAP and NSF bind to the core complex and break up the complex using the ATPase activity of NSF.

d) This is a working hypothesis. It's currently being tested in many labs around the world and there are different opinions on exactly when each of these interactions occurs with respect to the vesicle fusion event. This is currently the most popular model for how vesicle fusion takes place - but there's much more work to be done, before we'll really know. Figure 3. Figure 4.

C) Medicinal use of BoNT A: FDA licensed botulinum toxin in December 1989 for treating two eye conditions--blepharospasm and strabismus--characterized by excessive muscle contractions. It is now marketed under the trade name Botox.